Isolation and Identification of bacteria capable of degrading Naphthalene from sediments collected area near the Panama Canal

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ABSTRACT

Considering the increase in accidental oil spills occurring around the Panama Canal, and given the fact that few investigations have been conducted to the application of biodegradation in coastal marine environments, we developed the present study with the aim of isolating and identifying bacterial strains capable of degrading hydrocarbon fractions. The isolation was conducted with a minimal medium of mineral salts of 2.3% NaCl. The inoculation was performed inoculated with 5 g of sediment in 250 ml Erlenmeyer flasks with 0.055% naphthalene (the only carbon source), Successive transfers were made periodically to the new medium, and each of the cultures were incubated at 37°C with constant stirring at 150 rpm for 30 days in order to observe the characteristic turbidity of bacterial growth. All isolates were examined by Gram's staining reaction to differentiate between Gram positive and Gram negative bacteria. Over 8 strains, that were capable of using naphthalene as the only carbon and energy source for growth, were isolated from marine sediment samples. An automated test system API 20E was used for determination and identification of isolates. This study showed that two species of bacteria, Rhodococcus equi and Corynebacterium propinquum, that are able to sediments on naphthalene as a carbon and energy source, were isolated from the terrestrial and aquatic sites in the area near the Panama Canal. Our experiments demonstrated that naphthalene degrading microorganisms are not restricted to oil polluted sites.

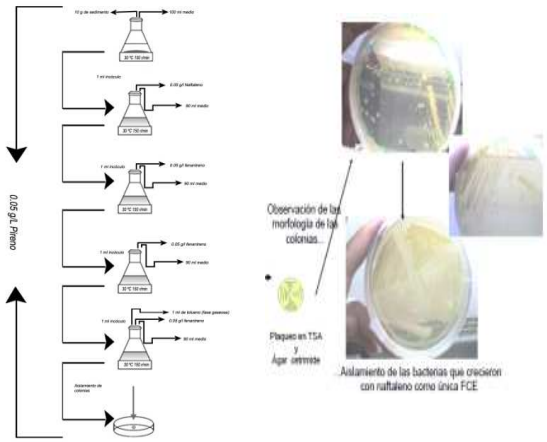
INTRODUCTION

One of the most important sources of environmental pollution worldwide is the discharge of petroleum hydrocarbons into aquatic and terrestrial ecosystems (Rahman et al. 2003). United States Environment Protection Agency has listed 16 PAHs (Polycyclic Aromatic Hydrocarbons) as priority pollutants because of their toxicity, mutagenicity and carcinogenicity (Keith and Telliard 1979). Extensive research has been conducted on the transportation and transformation of PAHs in natural environments, including water (Zhang et al. 2009a), soil (Thiele-Bruhn and Brümmer 2005) and sediment (Marcon et al. 2007).

MATERIALS AND METHODS

Naphthalene, purchased from Sigma–Aldrich, was used as the sole carbon source for enrichment of degrading bacteria. The bacterial consortium was isolated from a mixture of five different sampling sites in the area near the Panama Canal (Manzanillo Bay). The samples were obtained by dredging at depth of 12 meters. As stated before, isolation was conducted with minimal medium of mineral salts of 2.3% NaCl. The inoculation was performed with 5 g of sediment in 250 ml Erlenmeyer flasks with 0.055% naphthalene (the only carbon source), Successive transfers were made periodically to the new medium, and each of the cultures were incubated at 37 °C with constant stirring at 150 rpm for 30 days in order to observe the characteristic turbidity of bacterial growth. Once this period finished, 1 ml of each sample was taken in order to be inoculated on the plates containing the same medium solidified with agar. The growth was determined by the colorimetric method which was measured by spectrophotometry at 525 nm corresponding to an optical density (OD) of approximately equal to 2. Successive transfer were made periodically to the new medium (figure 1.).

Figure 1. Methodology used for the isolation of bacterial strains capable of degrading Polycyclic Aromatic Hydrocarbons



RESULTS AND DISCUSSION

The original naphthalene enriched mixed culture was obtained by adding 25 g of fresh sediment of the sample immediately after field collection. The mix was done with 150 ml sterilized medium minimum (MM) containing 50 mg/l of naphthalene in Erlenmeyer flasks, and shaken in an orbital shaker with 150 rpm at 30^{o}C in the dark. Two weeks later, 10 ml aliquots were transferred to 100 ml of fresh MM containing 50 mg/l of naphthalene, and the flasks were shaken for another two weeks. Bacterial colonies were selected by using as a criterion its potential to degrade the naphthalene. All isolates were examined by Gram's staining reaction to differentiate between Gram positive and Gram negative bacteria. All isolates were examined by Gram´s straining reaction (Table 1).

A total of 8 strains, that were capable of using naphthalene as the only carbon and energy source for growth, were isolated from marine sediment samples. The microorganisms were identified according to general principles of microbial classification, using selective media and microscopic examination of its morphological characters. An automated test system API 20E (BioMerieux) was used for determination and identification of the isolates. The bacteria were identified as **Rhodococcus equi and Corynebacterium propinquum (figure 2).**

Table 1: Characteristics of the strains found in the area near the Panama Canal

GRAM	MORPHOLOGY	COLONIES
(+)	Pleomorphic bacillus	Bluish entire edge
(--)	Sporulated bacillus	Yellow, elevated, wavy edge

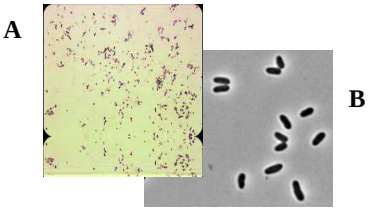


Figure 2. Identified bacteria by automated API 20 NE as a.) *Rhodococcus equi* b.) *Corynebacterium propinquum*

CONCLUSIONS AND FUTURE WORK

This study showed that two species of bacteria, which are able to sediments on naphthalene as a carbon and energy source, were isolated from the terrestrial and aquatic sites in the area near the Panama Canal. These bacteria were identified as *Rhodococcus equi* and *Corynebacterium propinquum*. Our experiments demonstrated that naphthalene degrading microorganisms are not restricted to oil polluted sites. This finding supports the fact that crude oil degrading microorganisms are widely distributed in the environment and, therefore, can be "easily" collected from sites with no apparent history of crude oil pollution, as it was in our case.

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